

Amendments to the Specification

**Please replace paragraph 040 spanning from page 12 to page 13 with the following amended paragraph:**

**[040]** To confirm the classification of this purified enzyme as a cathepsin L-like proteinase, a concentrated sample of pooled cathepsin-L1 from the gel filtration column was sequenced using an Applied Biosystems 477A protein sequencer. The resulting amino-acid sequence was aligned with previously determined sequences of known cathepsin-L molecules and the cathepsin-B sequence of Schistosoma mansoni (Table 1 below). Identical residues to those in F. hepatica cathepsin-L1 are shown by the dots. In the first 19 residues of the N-terminal sequence the fluke cathepsin-L has 63% identity with cathepsin-L molecules from both bovine and chicken liver sources, 53, 59 and 53% homology with cathepsin-L molecules from rat, human and Trypanosoma cruzi respectively, and 26% homology with a cathepsin-B from S. mansoni

	-1	1	2	3	4	5	6	7	8	9	10	11		12	13	14		15	16	17	18	19		
<u>F.hepatica</u> (C-L1)	A	V	P	D	K	I	D	P	R	E	S	G	-	-	Y	V	T	-	-	G	V	K	D	Q
<u>[SEQ. ID NO: 1]</u>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Bovine (C-L)	L	P	D	S	V	D	W	R	E	K	G	-	-	G	V	T	-	-	P	V	K	D	Q	
<u>[SEQ. ID NO: 2]</u>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Chicken	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
liver (C-L)	-	A	P	R	S	V	D	W	R	E	K	G	-	-	Y	V	T	-	-	P	V	K	D	Q
<u>[SEQ. ID NO: 3]</u>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Rat liver (C-L)	-	I	P	R	S	V	D	W	R	E	K	G	-	-	Y	V	T	-	-	P	V	K	D	Q
<u>[SEQ. ID NO: 4]</u>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Human	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
liver (C-L-	-	A	P	R	S	V	D	W	R	E	K	G	-	-	Y	V	T	-	-	P	V	K	D	Q
<u>[SEQ. ID NO: 5]</u>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
<u>T.cruzi</u> (c-p)	-	A	P	A	A	V	D	W	R	A	R	G	-	-	A	V	T	-	-	A	V	K	D	Q
<u>[SEQ. ID NO: 6]</u>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
<u>S.mansoni</u> (C-B)	-	I	P	S	N	F	D	S	R	K	K	W	P	G	C	K	S	I	A	T	I	R	D	Q
<u>[SEQ. ID NO: 7]</u>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	

Please replace paragraph 068 spanning from page 25 to page 26 with the following amended paragraph:

[068] By contrast with the two chain structure known to exist for mammalian Cathepsin L, the finding of a single N-terminal amino acid sequence for both Cathepsin L1 and L2 implies the presence of only a single chain. It is however possible that a second N-terminally blocked chain is present.

	-1	1	2	3	4	5	6	7	8	9	10	11		12	13	14	
<u>F.hepatica</u> (cathepsin L2) [SEQ. ID NO: 8]	A	V	P	D	K	I	D	R	R	E	S	G	-	-	Y	V	-
<u>F.hepatica</u> (cathepsin L1) [SEQ. ID NO: 9]	A	V	P	D	K	I	D	P	R	E	S	G	-	-	Y	V	T
<u>Chicken liver</u> (cathepsin L) [SEQ. ID NO: 10]	-	A	P	R	S	V	D	W	R	E	K	G	-	-	Y	V	T
<u>Rat liver</u> (cathepsin L) [SEQ. ID NO: 11]	-	I	P	R	S	V	D	W	R	E	K	G	-	-	Y	V	T
<u>Human liver</u> (cathepsin L) [SEQ. ID NO: 12]	-	A	P	R	S	V	D	W	R	E	K	G	-	-	Y	V	T
<u>Bovine spleen</u> (cathepsin S) [SEQ. ID NO: 13]	L	P	D	S	M	D	W	R	E	K	G	-	-	C	V	T	
<u>T.cruzi</u> (c-p) [SEQ. ID NO: 14]	-	A	P	A	A	V	D	W	R	A	R	G	-	-	A	V	T
<u>S.mansoni</u> (C-B) [SEQ. ID NO: 15]	-	I	P	S	N	F	D	S	R	K	K	W	P	G	C	K	S

**Please replace paragraph 069 spanning from page 26 to page 27 with the following amended paragraph:**

[069] The kinetic constants of F.hepatica cathepsins L1 and L2 enzymes were determined for 11 different substrates: Z-Phe-Arg-AMC, Bz-Phe-Val-Arg-AMC, Suc-Leu-Leu-Val-Tyr-AMC [SEQ. ID NO: 16], H-Leu-Val-Tyr-AMC, Tos-Gly-Pro-Lys-AMC, Tos-Gly-Pro-Arg-AMC, Boc-Val-Pro-Arg-AMC, Z-Arg-Arg-AMC, Z-Arg-AMC, Suc-Ala-Phe-Lys-AMC and Boc-Val-Leu-Lys-AMC. One mg of fluorogenic substrate was dissolved in 100  $\mu$ l of dimethyl formamide. This stock solution was diluted in 0.1M glycine pH 7.0 to achieve the desired concentration of substrate. Each substrate concentration was in triplicate and the final assay volume was 1.0 ml. Included in the 1ml aliquot was 20 $\mu$ l of enzyme and 50 $\mu$ l of 10mM dithiothreitol. The samples were incubated at 37°C for 30 min before stopping the reaction with the addition of 200 $\mu$ l of 1.7 M acetic acid. The samples were assayed for released 7-amino-methyl-coumarin as above. The kinetic constants  $V_{max}$  and  $K_m$

were obtained by non-linear regression according to the method of Barrett et al (Biochem J. 201, p.189-198) except that 20µl of cathepsin L2 was incubated with 20µl of 1.0µM - 0.1µM E-64 in a final volume of 80µl 0.1M glycine pH 7.0 for 30 mins at 37°C. Twenty µl of 1/10 cathepsin L1 was incubated with 20µl of 5µM - 0.5 µM E-64 in a final volume of 80µl 0.1M glycine pH 7.0 for 30 min. at 37°C. All of the incubated sample was assayed for the fluorogenic substrate Z-Phe-Arg-AMC as above.

**Please replace paragraph 071 on page 29 with the following amended paragraph:**

**[071]** DNA sequences were obtained by amplification of F.hepatica cDNA using conventional polymerase chain reaction (PCR) techniques. The sequences are shown in Figures 6-8 [Figure 6:nucleic acid:SEO. ID NO: 17; amino acid:SEO. ID NO: 18; Figure 7:nucleic acid:SEO. ID NO: 19; amino acid: SEO. ID NO: 20; Figure 8:nucleic acid:SEO. ID NO: 21; amino acid:SEO. ID NO: 22] with the amino acids for which they code. Genomic DNA from other helminth parasites was probed with the F.hepatica sequence JDCLONEC shown in Figure 6 using the Southern blotting technique and conditions of moderate stringency. Bands were observed in the heartworm (*Dirofilaria immitis*) and blowfly (*Lucilia cuprina*) channels indicating respectively strong and weak hybridisation.